## RESPONSE

### I. Status of the Claims

Claim 8 is presently pending. No prior art rejections are presently of record. Claim 8 has been amended to better clarify the invention and correct a typographical error. Support for the amendment of Claim 8 can be found at page 6, line 15 (the first sentence of Section 5). The amendments are not deemed to constitute new matter.

### II. The Present Claims Are Patentable And The Rejections of Record Should Be Withdrawn.

#### a) Rejections Under 35 U.S.C. § 101 and §112, First Paragraph

The Examiner's rejections of Claim 8 under 35 U.S.C. section 101 and the intertwined rejections under section 112, first paragraph are respectfully traversed. The Examiner has apparently adopted the position that the claimed invention lacks patentable utility due to its not being supported by either a specific and/or substantial utility or a well established utility.

When cells exemplary of the described ES cell line were used to generate mice homozygous for the mutation that was genetically engineered into the novel gene at issue (a related human version of which is described in PCT application no. WO200061623), the mice exhibited marked resistance to asthma-inducing immune challenge. Such animals are specifically contemplated in the second to last paragraph of the Summary of the Invention (at Page 5), and the use of the broader invention to investigate asthma, *inter alia*, is disclosed in the specification near the bottom of the fourth full paragraph of Section 5.5 (line 31 of page 37 of Applicants copy– which may differ from the PTO copy in view of it potentially being printed on a different printer prior to filing). In view of the clear phenotype/medical discovery attributable to the described ES cells, little question can remain about that the described ES cell line has a substantial and specific "real world" utility.

Applicants have amended Claim 8 to replace the word murine with mouse. Accordingly, the Examiner's rejection of Claim 8 under 35 U.S.C. section 112, first paragraph is deemed to have been avoided by amendment. In view of the above amendments and remarks, the Examiner is respectfully requested to withdraw the rejection of Claim 8 under 35 U.S.C. §101 and 35 U.S.C. §112, first paragraph.

Applicants would also like to address certain apparent misconceptions about the present invention. For example, the Examiner seems to imply that the present invention cannot have utility because the functional properties of the (mutated) sequence are not known. Determining the physiological function of the targeted gene is a major point of the present invention. If the function of the sequence was actually already known, why do the experiment?

Regarding the Examiner's contention that the broader utility of the invention (the application describes over 1,000 different ES cell clones) is not well established because of a broader lack of specific utility, the Examiner is respectfully requested to consider that the novel mutated ES cell lines at issue are each *specifically* identified by corresponding exon sequences (presented in the Sequence Listing) that provide *a unique (and hence, highly specific) resource* for mapping that portion of the murine genome that encodes the described exon sequence and, by proxy, that portion of the human genome that encodes the human ortholog of the described sequences. More importantly, the specification teaches that genetically engineered mutation present in the specifically described mouse ES cell clone is designed to disrupt the normal function of the mutated allele. The specification also teaches that the otherwise totipotent ES cells can be used to generate animals that specifically lack the function of the disrupted gene. As specifically discussed for the asthma resistant animals discussed above, animals produced from the various clones identified by sequence tag in the Sequence Listing provide a novel resource for specifically determining the physiological role of the mutated gene. Accordingly, by using the specifically described mutated ES cells and following the teaching in the specification, the present invention broadly enables determinations of mutated gene function in the broader context of mammalian physiology. More importantly, in those instances, such as that specifically described above, where the observed phenotype defines an overt target for the development of therapeutic agents, the present application broadly enables the precise type of demonstrated pharmaceutical utility contemplated by U.S. patent law (and is also exemplary of the type of specific and substantial and well-established utility that even satisfies the substantially more onerous U.S. Patent office utility guidelines). Given that there can be no question that the "final product"of the presently claimed invention has already been used to identify a substantial pharmaceutical utility, there can be no question that the presently described mouse ES cell line also has a substantial, credible, and well established utility.

From a broader policy perspective, the Examiner is also requested to consider that public and private efforts have spent several billion dollars to obtain human genomic sequence data (and that corporate partners have committed to spending millions of dollars for early access to human genomic sequence), one can state that such genomic sequence data, in part or in whole, have a demonstrated, substantial, and specific utility fully within that contemplated by 35 U.S.C. section 101 (see also the issues of "Nature" (2001, *409*:745-964) and "Science" (2001, *291*:1304-1351 that were both dedicated Human genomic sequence data). The practical implementation of the present invention adds value to human genomic data by assigning critical functional annotation to the human sequence data. It is thus axiomatic that an invention that adds value to an asset having *demonstrated and substantial economic and scientific utility* must also have substantial utility. As such, it should be clear that even in the broader context of the application as a whole (as opposed to the specifically claimed ES cell line), the presently described invention has a demonstrated substantial utility. Moreover, each of the specifically described mouse ES cell clones provide a *specific* resource for discovering the *in vivo* function of a *specific* human ortholog.

To the extent that the Examiner may question the broader utility of knockout animals (many of which form the basis of issued U.S. Patents), the Examiner is respectfully reminded that the legal standard for establishing the utility of an invention necessarily focuses on whether those skilled in the art would find the applications assertion of utility to be credible. Applicants provide below conclusive evidence that the scientific community accepts the broader utility of the described technology. The 2001 Lasker award, arguably the most prestigious scientific award presented in the United States, was presented to three pioneers of mouse ES cell technology. Reproduced below for the Examiner's convenience are the comments by Lasker award presenter Ira Herskowitz which aptly provide a context for the present invention and the scientific community's acceptance of the real world significance of the broader invention:

> Albert Lasker Award for Basic Medical Research, 2001
> Comments at the Awards ceremony
> Presented by Ira Herskowitz
>
> "The release of the human genome sequence in draft form makes this a landmark year in the history of biology. Now we know that we have 30,000 or so genes (or is it 50,000?). We are

now faced with several important questions, which include:

First, what are the functions of these genes and the proteins that they code for? And, second, how can we use this information to improve human health?

Until the ability to knock out genes in the mouse was developed, determining the function of human genes seemed largely out of reach, tantalizingly so. For example, we might know of a human protein that is found only in certain cells of the brain and suspect what it might do, but how can we find out? Or, we might know of a gene in the fruitfly that is necessary for its development and see that humans have a very similar gene. Does it perform a similar function in humans? A powerful way to link a gene to function is to study the behavior of a mutant that lacks that gene and then see what the mutant can and cannot do. It's somewhat like disabling an automobile by removing one part and then inferring the function of the part that was removed. But we can't knock out genes in a human, so how can such mutants be produced?

The mighty mouse has come to the rescue. Its genes are typically 95% identical in sequence to ours, and we share the vast majority of our genes with the mouse. Despite the obvious differences between human and mouse in morphology and in some physiological processes, these differences are greatly outweighed by our similarities: they have kidneys and brains like ours; they have an immune system and develop a lot like humans; and they get diseases such as cancer and others that affect their cardiovascular and nervous systems like us. In some respects, mice are "pocket-sized humans". The bottom line is that the mouse provides the opportunity, dreamed about for decades, to make the link between a mammalian gene and its function. How is this done?

Building on more than one hundred years of genetic and embryological studies of the mouse, **Mario Capecchi, Martin Evans,** and **Oliver Smithies** have created a magic wand by which it is possible to modify any mouse gene with exquisite precision -- to completely delete it or to produce a specifically altered form of the gene.

The same technology also makes it possible to go the other direction - instead of knocking out a mouse gene, it's possible to restore function to a gene that is defective.

Let's now look at the process by which a mouse knockout is constructed.

A key piece of starting material is a mouse gene that's already been cloned: it might be a mouse gene corresponding to a human gene or a mouse gene corresponding to a fruitfly or nematode gene. The goal is to construct a mouse that lacks this gene. The second key piece of starting material is a special mouse cell line where the gene is going to be knocked out.

There are three steps for constructing a mouse knockout. In the first, a cloned gene is manipulated in a test tube to delete all or part of the gene. This is routine molecular biology. In step two, the mutated DNA is introduced into special mouse cells, where the mutated DNA replaces a normal gene copy in the chromosome. The crucial aspect of this process is that the mutant gene has to find the related sequences in the chromosome, so-called homologous DNA sequences, and then undergo recombination to switch places with the good gene. The ability

of the introduced DNA to find the homologous DNA sequences is called "gene targeting". There was no evidence for gene targeting in animal cells growing in culture and great doubt about whether this could be done. This is where Capecchi and Smithies made their most important contributions. In the third step, the cells with the targeted, inactivated gene are grown into a mouse that has this inactivated gene. It was Martin Evans who isolated the cell lines that made this possible and showed that genetic changes introduced into these cells in culture could be transmitted through the germ line and into mutant, progeny mice.

Let's now look at our awardees.

Verona, Italy has given us not only Romeo and Juliet, but Mario Capecchi. His early days as a child included living in orphanages and on the street in war-torn Italy from 4-9 years of age, then growing up in a nurturing Quaker environment in Pennsylvania. I refer interested people to articles that are available on the Internet. Capecchi did his graduate work at Harvard with Jim Watson and was enormously productive, making textbook discoveries on molecular mechanisms underlying protein synthesis. This was a golden age of molecular biology. Mario learned his lessons well, and when he established his own laboratory at the University of Utah in 1973, he sought to bring molecular genetics to animal cells growing in culture and learn how to manipulate the genes of these cells. This led him to undertake a series of studies beginning in 1977 that demonstrated gene targeting in animal cells and culminated in the construction of one of the first knockout mice in 1989. His first indications of homologous recombination in animal cells were published in 1982 and fueled a series of logical and remarkable studies that provide the standard methods for knocking out mouse genes.

Oliver Smithies was trained as a biochemist, but throughout his scientific career, homologous recombination kept on cropping up, and he came to think about how it could be used to fix defective genes. Smithies was born in Halifax, England and raised in the United Kingdom. After studying at Oxford University, he came to the University of Wisconsin for postdoctoral studies in 1951 and was on the faculty there for 28 years, from 1960-1988. He is presently at the University of North Carolina at Chapel Hill, and may well have flown here in his own little plane to attend this luncheon. After important early contributions springing from his development of a method for fractionating proteins, he became intrigued by the structure and evolution of mammalian genes, which meant that he became involved in cloning these genes.

In the early 1980s, Smithies began to wonder whether homologous recombination - gene targeting - could be carried out experimentally to correct a defective gene, for example, a mutant globin gene. For this type of genetic correction to occur, exogenously introduced DNA would have to target to the homologous chromosomal DNA sequence and recombine with it. But was this possible? No one had demonstrated gene targeting in animal cells.

In 1985 Smithies and colleagues demonstrated that they could introduce a DNA segment containing part of the globin gene into cells and then find cells in which this DNA segment had targeted to the chromsomal globin gene. This was a tour-de-force of sophisticated molecular genetics. His strategy was completely different from that used by Capecchi and though laborious, the demonstration of targeting was unequivocal.

These studies from the Capecchi and Smithies laboratories provided one of the essential ingredients for constructing gene knockouts in mice, the ability to target genes in cultured animal cells. The crucial next step was to take mouse cell lines modified in this manner and produce mice from them.

The history of mammalian embryology is intellectually rich and filled with great practical applications. It was nurtured by the agricultural industry among others and involved important work with rabbits and mice. The UK can lay claim to many important contributions in this area, and thus Martin Evans is part of a distinguished tradition. Evans was born in the UK and graduated from Cambridge in 1963. He then went to University College London, where he studied vertebrate development using frogs. After working with a certain type of cancer cell line that could differentiate in cell culture and be used to generate whole mice, Evans set out to isolate normal cells from an early mouse embryo that would have similar properties. Work from Richard Gardner argued for the existence of such cells, but culturing them had been elusive. In 1981, Martin Evans and Matt Kaufman and, independently, Gail Martin, in the U.S. were successful in isolating such cells, which have become known as embryonic stem cells, "ES cells". Evans then carried out an important series of experiments with his students Allan Bradley and Elizabeth Robertson that demonstrated that these ES cells could contribute to the mouse germ line. They further showed that genetically manipulated ES cells could transfer their genetic changes to progeny mice. The importance of ES cells was immediately recognized by Capecchi and Smithies, who learned how to grow ES cells and demonstrated that they could carry out targeted genetic alterations with them.

The first knockout mice constructed by gene targeting were published in 1989, and the rest is history. More than 4,000 different knockout mice have been constructed in the last dozen years, and many more are in the works! To keep on top of this fast-moving field, I suggest looking at the Jackson Laboratory's website, where you can find columns called "It's a Knockout!" and "KO of the Month".

The ability to modify the genetic make-up of a mouse by design provides a wealth of information on the function of the gene that is knocked out. Every aspect of mammalian physiology is being penetratingly analyzed by this technique. Particularly notable are the discoveries made on how the immune system functions, which have enormous implications for human health. Knockout mice made it possible to demonstrate unequivocally the molecular basis for prion diseases such as mad-cow disease. Knockout technology is also used to create mice that have versions of human diseases such as cystic fibrosis, muscular dystrophy, atherosclerosis, and many others. These mice make it possible to follow the course of a disease and provide an opportunity to identify and test drugs to ameliorate or cure these diseases.

The ability to precisely tailor mouse genes has completely revolutionized the practice of biomedical science for the last decade and is likely to become even more important in the decades to come. We are certain to reap an enormous bounty of information from knockout mice and reap great benefits for the improvement of human health."

In view of the above remarks, there should be no doubt that there is ample evidence of record that those skilled in the art would find that the mutated ES cell clones described in the present application have both a well established and credible utility. For a technical point of reference, the technology used to generate the presently described mutated clones has, in just a few years, produced and identified many fold more identified and characterized ES cells clones than have cumulatively been produced to date *by the world-wide scientific community* using the technologies that were the subject of the 2001 Lasker award referenced above.

In the event that the Examiner may still have some lingering doubts, Applicants invite the Examiner to further consider the guidance of the National Institute of Health which issued a request for applications entitled TOOLS FOR INSERTIONAL MUTAGENESIS IN THE MOUSE on January 25, 2001 (RFA-DA-01-011 which stated in part:

> "This RFA solicits proposals for development of tools and techniques for the establishment of random and targeted sequence-tagged insertion libraries of embryonic stem (ES) cells that can be used to generate mutant mice in which the expression of the tagged gene could be controlled temporally and spatially. The development of such a resource for wide distribution to the scientific community would make it possible to scan the sequence database for any gene of interest and order the corresponding targeted ES cell line. Ideally, the insertional mutagenesis system developed would permit a wide range of genetic analyses and manipulations, including enhancer-trapping, conditional knockouts, conditional expression or overexpression, etc. It also would permit the larger community of investigators to utilize genomic resources efficiently. This effort complements ongoing National Institutes of Health (NIH) efforts to create and characterize induced point mutations in mice using ethylnitrosourea (ENU) and provides a functional genomics tool to translate the information from the Mouse Genome Sequencing Project. Further information about NIH initiatives on mouse genomics and genetics resources is available at http://www.nih.gov/science/mouse."

In brief, that branch of the U.S. government that is specifically tasked with sponsoring technologies having high biomedical utility, has already financially "validated" the utility of a related, albeit technically inferior, gene trap technology by providing many millions of dollars of funding for such efforts. In view of the direct governmental validation of biomedical utility of gene trapped mouse ES cell clones, one cannot credibly assert that the presently described ES cell clones somehow lack a well-established utility.

Finally, Applicants would like to turn the discussion from the utility inherent in the scientific merits of the broader invention, to a nuts-and-bolts practical/industrial utility for the described ES cell clones. In addition to their generation and study, the storage, handling, and transfer of genetically

engineered mice is an expensive endeavor. Unlike most academic facilities, industrial vivariums are typically run under pathogen-free "barrier" conditions and extensive efforts are undertaken to protect the pathogen free integrity of the system. Consequently, new colonies being brought into the barrier are often "rederived" (often using from ES cells) into the barrier via birth from "clean" surrogate mothers animals. Additionally, space in such facilities is often at a substantial premium. Unlike live animals, mutated ES cell clones can be stored in liquid nitrogen. Tens of thousands of mutated ES cell clones can be stored in a couple of liquid nitrogen freezers whereas many hundreds of thousands of square feet of "barrier" vivarium space would be necessary to store corresponding numbers of live mutant animal colonies. From a purely practical perspective, a microtiter plate or two roughly corresponds to an entire room of vivarium space (the absence of such practical efficiencies largely contributed to the broader failure of the NIH's efforts to approach attack gene function through ENU mutagenesis in live mouse colonies). In brief, although the biotech utility analysis typically focuses on scientific nuances (and more often scientific metaphysics where sequence data are concerned), the practical savings and efficiencies of working with ES cell clones provide an industrial utility that is not dissimilar from the efficiencies obtained between storing paper files as compared to digital data storage– an industrial utility clearly recognized by the U.S. Patent Office as evidenced by its recent adoption of electronic document storage. Accordingly, there can be no question of the industrial utility of the more broadly described invention.

In view of the overwhelming evidence of the substantial, credible, specific, and well-established utility of the presently claimed invention, and in view of the absence of any evidence of record specifically refuting the utility of the described ES cell clones, the Applicants' respectfully request that the Examiner withdraw the pending rejection of Claim 8 under 35 U.S.C. section 101 as well as the related rejections under 35 U.S.C. section 112, first paragraph.

**b) Rejections under 35 U.S.C. Section 112, Second Paragraph**

The Examiner has rejected Claim 8 under 35 U.S.C. section 112, second paragraph for allegedly being indefinite over the recitation "first disclosed in" and the "an engineered mutation....comprising a polynucleotide sequence...". The Examiner's rejection has been avoided in part by amendment and the is partially and respectfully traversed. Applicants have amended Claim 8 to remove the term "first disclosed in." Regarding the comprising language, Applicants reading of Claim

8 reveals that the engineered mutation is present in the gene that normally comprises (*i.e.*, "encodes" a transcript containing) the spliced exon sequences shown presented in SEQ ID:705). Those skilled in the art would understand that, in the context of the described invention, the "mutation" corresponds to any and all mutations that disrupt the functional expression of the endogenous gene (in the present instance, gene disruption was confirmed by the absence of wildtype transcript and the observed immune phenotype. In view of these remarks and amendments, the Examiner is respectfully requested to withdraw the pending rejection of Claim 8 under 35 U.S.C. §112, second paragraph.

To the extent that the Examiner might suggest alternative claims language that would avoid any of the above amendments, the Examiner is invited to suggest such language if it will put the claim or claims in condition for allowance.

## III. CONCLUSION

In view of the foregoing amendments and remarks, the Applicants believe that the application is in good and proper condition for allowance. Early notification to that effect is earnestly solicited.

If the Examiner feels that a telephone call would expedite the consideration of the application, the Examiner is invited to call the undersigned attorney at (281) 863-3333. The Commissioner is authorized to charge any underpayment or credit any overpayment to Deposit Account No. 50-0892 for any matter in connection with this response, including fees for any extension of time, which may be required.

Respectfully submitted,

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Date

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